## **CLAIM AMENDMENTS**

1.-32. (canceled)

33. (thrice amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

<u>creatine</u> +  $H_2O$  → <u>sarcosine</u> + <u>urea</u>

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point of 4.5.

34. (canceled)

35. (thrice amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine +  $H_2O \rightarrow sarcosine + urea$ 

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

36. (thrice amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H<sub>2</sub>O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

37. (thrice amended) A creatine amidinohydrolase (i) encoded by a nucleic acid sequence obtained by mutating (a) the nucleic acid sequence of SEQ ID NO:2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:1 and (ii) having the following physicochemical properties:

Action: catalyzing the following reaction:

<u>creatine +  $H_2O$  → sarcosine + urea</u>

Km values for creatine in a coupling assay using a sarcosine oxidase and a

peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0-9.0 (at a temperature of about 37° C)

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

- 38. (once amended) A method for producing the creatine amidinohydrolase of claim 33, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.
- 39. (once amended) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 33, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.
- 40. (once amended) A method for determining creatine in a sample, which comprises measuring absorbance of a pigment produced by the reaction of the reagent of claim 39 with the sample.
- 41. (once amended) A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 33, a

sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

- 42. (once amended) A method for determining creatinine in a sample, which comprises measuring -absorbance of a pigment produced by the reaction of the reagent of claim 41 with the sample.
- 43. (once amended) A method for producing the creatine amidinohydrolase of claim 35, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.
- 44. (once amended) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 35, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.
- 45. (once amended) A method for determining creatine in a sample, which comprises measuring -absorbance of a pigment produced by the reaction of the reagent of claim 44 with the sample.
- 46. (once amended) A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 35, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.
- 47. (once amended) A method for determining creatinine in a sample, which comprises measuring -absorbance of a pigment produced by the reaction of the reagent of claim 46 with the sample.
- 48. (twice amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine +  $H_2O \rightarrow sarcosine + urea$ 

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

K<sub>m</sub> value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:

<u>3.5 - 10.0 mM</u>

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

49. (twice amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

 $\underline{\text{creatine} + \text{H}_2\text{O} \rightarrow \text{sarcosine} + \text{urea}}$ 

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

 $\underline{K_m}$  value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:

 $4.5\pm1.0 \text{ mM}$ 

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

- 50. (once amended) The creatine amidinohydrolase of claim 49, which is obtained from *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375).
- 51. (twice amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine + H<sub>2</sub>O → sarcosine + urea

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

 $K_m$  value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:  $6.5\pm1.0$  mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

- 52. (once amended) The creatine amidinohydrolase of claim 51, which is obtained from *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374).
- 53. (twice amended) A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction;

creatine +  $H_2O \rightarrow sarcosine + urea$ 

Optimum temperature: about 40-50° C (at pH of about 7.5)

Optimum pH: pH about 8.0 - 9.0 (at a temperature of about 37° C)

 $K_m$  value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase:  $9.0\pm1.0 \text{ mM}$ 

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 4.5.

- 54. (once amended) The creatine amidinohydrolase of claim 53, which is obtained from *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).
- 55. (not amended) A method for producing the creatine amidinohydrolase of claim 48, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.
- 56. (twice amended) The method of claim 55, wherein said microorganism is selected from the group consisting of *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374), *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375), and *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).
- 57. (not amended) A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 48, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.
- 58. (not amended) The reagent of claim 57, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore, and a buffer.
- 59. (not amended) The reagent of claim 58, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase, and myeloperoxidase.
- 60. (not amended) The reagent of claim 58, in which the chromophore comprises a hydrogen receptor and a coupler.
- 61. (not amended) The reagent of claim 60, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

- 62. (not amended) The reagent of claim 60, in which the coupler is an aniline derivative or a phenol derivative.
- 63. (once amended) A method for determining creatine in a sample, which comprises measuring absorbance of the pigment produced by the reaction of the reagent of claim 57 with the sample.
- 64. (once amended) A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 48, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.
- 65. (not amended) The reagent of claim 64, in which the composition for the detection of hydrogen peroxide comprises and enzyme having a peroxidase activity, a chromophore, and a buffer.
- 66. (not amended) The reagent of claim 65, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase, and myeloperoxidase.
- 67. (not amended) The reagent of claim 65, in which the chromophore comprises a hydrogen receptor and a coupler.
- 68. (not amended) The reagent of claim 67, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.
- 69. (not amended) The reagent of claim 67, in which the coupler is an aniline derivative or a phenol derivative.
- 70. (once amended) A method for determining creatinine in a sample, which comprises measuring absorbance of the pigment produced by the reaction of the reagent of claim 64 with the sample.

Please cancel claims 71-75.